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## CHEMICALLY BONDED STATIONARY PHASES ON DIATOMITE SUPPORTS FOR USE IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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### SUMMARY

Chemically bonded stationary phases for use in liquid chromatography can be prepared by the reaction between appropriate silane monomers or partially polymerized silanes and functional groups on the surface of chromatographic support materials. Methods reported so far mostly concern the preparation of bonded phases on porous silica gel or superficially porous silica beads. In this study a variety of silane monomers was polymerized and chemically bonded to the surface of a totally porous diatomite support of a small particle size. The resulting packing materials were non-extractable and performed well in liquid chromatography.

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### INTRODUCTION

In conventional liquid-liquid chromatography some practical problems arise from the solubility of the stationary liquid in the eluent. Moreover, the stationary liquid may be stripped off mechanically at high flow velocities. These problems may be circumvented using chemically bonded stationary phases<sup>1</sup>. The reactivity of silanol groups on the surface of the usual chromatographic support materials offers a number of possibilities for the synthesis of chemically bonded phases. A review of the reactions and their practical application in the preparation of various column packings for liquid chromatography has been given by Locke *et al.*<sup>2,3</sup>. Silica materials, either totally porous silica gel or superficially porous spherical beads, are used almost exclusively as solid supports for bonded phase packings in liquid chromatography<sup>3</sup>. A number of workers<sup>4-9</sup> investigated bonding of stationary phases on diatomaceous earth supports (*e.g.*, Chromosorb, Celite). In these experiments chlorosilanes or alkoxy silanes were used with a small amount of water which permits polymerization as well as bonding to the surface of the solid support. Abel *et al.*<sup>7</sup> reported the polymerization of trichlorosilanes on the surface of Celite. Stewart and Perry<sup>8</sup> and Bossart<sup>9</sup> pointed out the application of these packing materials for liquid chromatography. Aue *et al.*<sup>4-6</sup> reported the preparation of chemically bonded silicones from apolar di- and

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trichlorosilanes. Prolonged continuous extraction was used to distinguish bonded from non-bonded silicone polymers. Pretreatment of the solid support was needed to obtain a chemically bonded liquid phase. However, the procedure described by these authors cannot be applied to the preparation of small-particle-size packings, as required for high-performance liquid chromatography.

It was demonstrated that good columns for liquid chromatography can be prepared from a small-particle-size diatomite support coated with conventional stationary phases<sup>10</sup>. These packings can easily be prepared in size fractions from an inexpensive starting material<sup>11</sup>. Therefore, it was tempting to investigate the synthesis of chemically bonded phases on this solid support material. Considering the low surface reactivity of diatomaceous earth the most suitable approach for obtaining reasonable loadings of bonded material seemed to be the combination of polymerization and surface-bonding reactions using appropriate silane monomers.

## EXPERIMENTAL

### *Surface treatment*

Hyflo Super Cel, particle size 7–11  $\mu\text{m}$ , was used as solid support. It was refluxed with concentrated hydrochloric acid, washed with distilled water until neutral, and dried in vacuum at 80°. In some cases the dry packing material was treated with a warm solution of silicon tetrachloride in purified dioxane. The latter treatment aims to increase the surface reactivity of the solid support. The unreacted silicon tetrachloride was removed by distillation. The chlorinated support was stored in dioxane under dry nitrogen.

### *Reaction with organosilanes*

Chemically bonded stationary phases have been prepared from chlorosilanes as well as from methoxysilanes. Chlorosilanes were reacted with the solid supports for 2 h in a boiling solution of dioxane with addition of a small quantity of water. The solvent was removed by evaporation and the resulting dry material was treated for 1 h at 100° to complete polymerization and chemical bonding. Methoxysilanes were hydrolysed and subsequently polymerized and bonded in dioxane solutions containing an excess of water and a suitable catalyst. After evaporation of the solvent, polymerization and bonding reactions were completed by heat treatment.

All packing materials were extracted with benzene in a hot Soxhlet apparatus for at least 24 h. After extraction the organic carbon content of the packing was determined. The experiments reported in this paper were carried out primarily in order to show the possibility of the preparation of bonded phases on diatomaceous earth for use in high-performance liquid chromatography; no systematic attempts were made to find out optimum reaction conditions.

### *Liquid chromatography*

The apparatus for liquid chromatography was constructed in our laboratory and has been described elsewhere<sup>11</sup>. Columns were made from thick-walled, precision-bore straight glass tubes with an internal diameter of 4 mm. The columns were packed by means of a simplified slurry technique. Samples were injected by syringe or high-pressure sampling valve (Chromatronix, Berkeley, Calif., U.S.A.).

## RESULTS AND DISCUSSION

A number of packings were prepared in order to test their chromatographic properties. Exhaustive extraction was used to remove non-bonded polymer. The results for some representative experiments are shown in Table I. The loading was calculated as 100 times the ratio of the weight of the silicone polymer represented as  $(R-Si-O_{3/2})_n$  over the total weight of the polymer and the solid support. The weight of the chemically bonded polymer was calculated from the organic carbon content of the extracted material.

TABLE I  
POLYMER LOAD FOR SOME PACKINGS

No.	Liquid phase monomer	Pretreatment	% bonded phase	% of theoretical phase
1	Amyltrimethoxysilane	Reflux conc. HCl	4.8	80
2	Amyltrimethoxysilane	Reflux conc. HCl	9.6	70
3	$\gamma$ -Glycidoxypropyltrimethoxysilane	Reflux conc. HCl	6.9	75
4	$\gamma$ -Glycidoxypropyltrimethoxysilane	Reflux conc. HCl	12.4	65
5	$\beta$ -(3,4-Epoxy-cyclohexyl)ethyltrimethoxysilane	Reflux conc. HCl	6.8	75
6	Octadecyltrichlorosilane	Reflux conc. HCl	4.2	40
7	Octadecyltrichlorosilane	Reflux conc. HCl + SiCl <sub>4</sub>	8.1	85

Good results were obtained in the preparation of bonded phases from alkoxy-silanes, judging from the amount of extractable polymer. The relative extractability of the organic material increases with increasing load. The packing materials, however, were slightly agglomerated, resembling small-porous diatomite particles coated with conventional stationary phases. A partial disintegration of the agglomerated particles was observed when a slurry of the packing was placed in an ultrasonic vibrator. The remaining agglomerated particles were removed by sedimentation. The agglomeration was not observed if a coarse-mesh support was used.

A satisfactory amount of chemically bonded polymer was also obtained from trichlorosilanes. An appreciable lower extractability resulted from silicon tetrachloride pretreatment of the support and the packing material did not show any agglomeration. The presence of water during the treatment of the solid support with a solution of the chlorosilane seems to be essential; if water is excluded in this step<sup>8</sup>, the organic material is found to be completely extractable. This result confirms the findings of Hastings *et al.*<sup>5</sup> It may be concluded from the absence of agglomeration that a more even distribution of the polymer on the surface was obtained for the trichlorosilane monomer, and this would result in excellent chromatographic properties of the packing material.

In liquid chromatography the distribution coefficient can be calculated from the ratio of the product of the activity coefficient of the compound in the mobile phase and the molar volume of the mobile phase to the product of the activity coefficient and molar volume for the stationary phase. It is obvious that a reasonable value

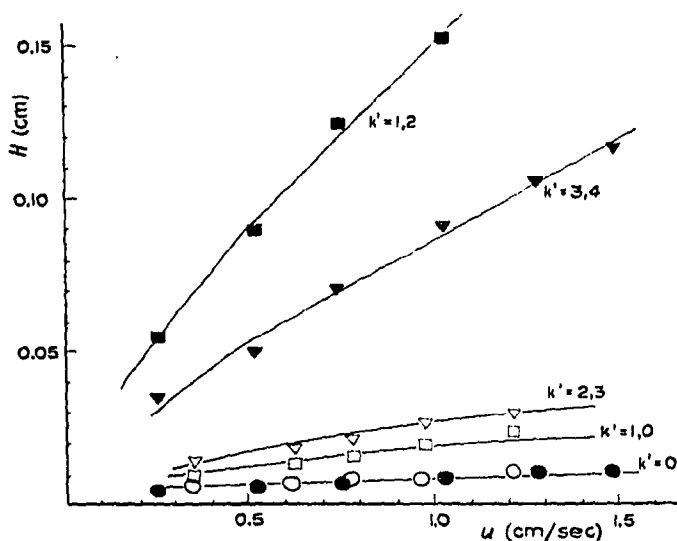


Fig. 1. Effect of carrier velocity,  $u$ , on plate height,  $H$ , for a bonded phase packing prepared from  $\beta$ -(3,4-epoxycyclohexyl)ethyltrimethoxysilane on Hyflo Super Cel, 7-11  $\mu\text{m}$ .  $\bigcirc$ ●, Chlorobenzene;  $\square$ ■, 2,4-dimethylphenol;  $\nabla$ ▼, *p*-cresol. Eluents:  $\bullet$ ■▼, *n*-heptane;  $\circ$ □▽, *n*-heptane + 2% ethanol.

of the distribution coefficient requires a high value of the quotient of the activity coefficients in the case of a high-molecular-weight stationary phase. Therefore very polar solvents should be chosen for apolar stationary phases and, inversely, very apolar carriers for polar stationary liquids. Kirkland<sup>12</sup> pointed out that poor column efficien-

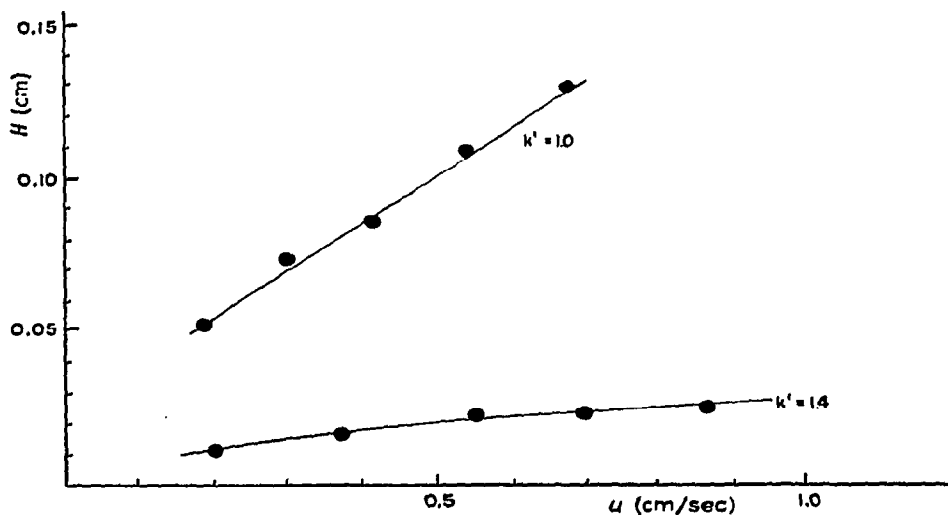


Fig. 2. Effect of carrier velocity,  $u$ , on plate height,  $H$ , for 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane on a bonded phase packing prepared from octadecyltrichlorosilane on Hyflo Super Cel, 7-11  $\mu\text{m}$ . Eluent: water-methanol (25:75) + 1% isooctane ( $k' = 1.4$ ) and water-methanol (25:75) ( $k' = 1.0$ ).

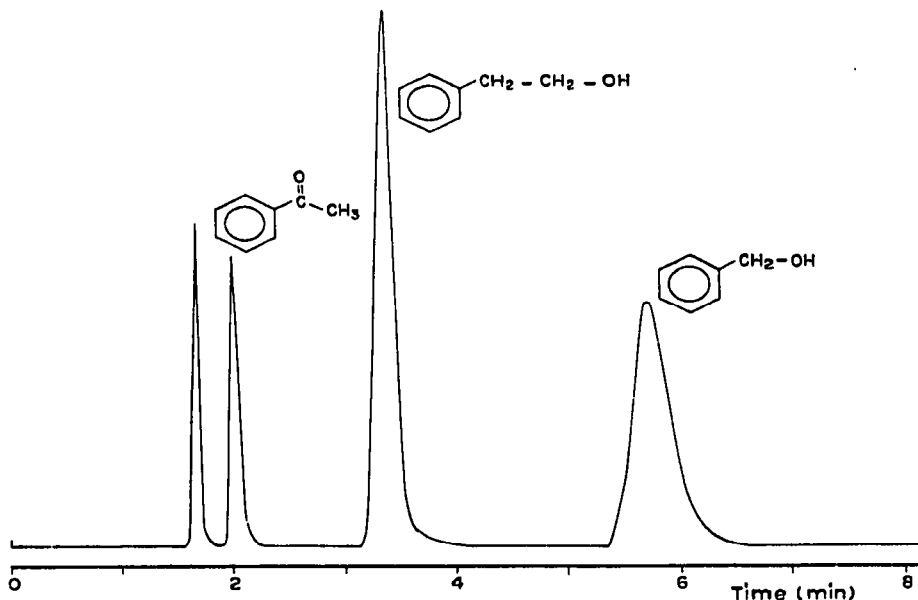


Fig. 3. Separation of a model mixture on a 20 cm  $\times$  4 mm column packed with a bonded phase prepared from  $\gamma$ -glycidoxypropyltrimethoxysilane on Hyflo Super Cel, 7–11  $\mu$ m. Eluent: isooctane.

cy may result from these extreme polarity differences, probably in consequence of a low diffusion rate in the polymer layer. This hypothesis is confirmed by the plate height curves given in Fig. 1 for *n*-heptane as eluent. The influence of the capacity ratio,  $k'$ , on the plate height with a maximum value for  $k' \sim 1$  points to slow mass

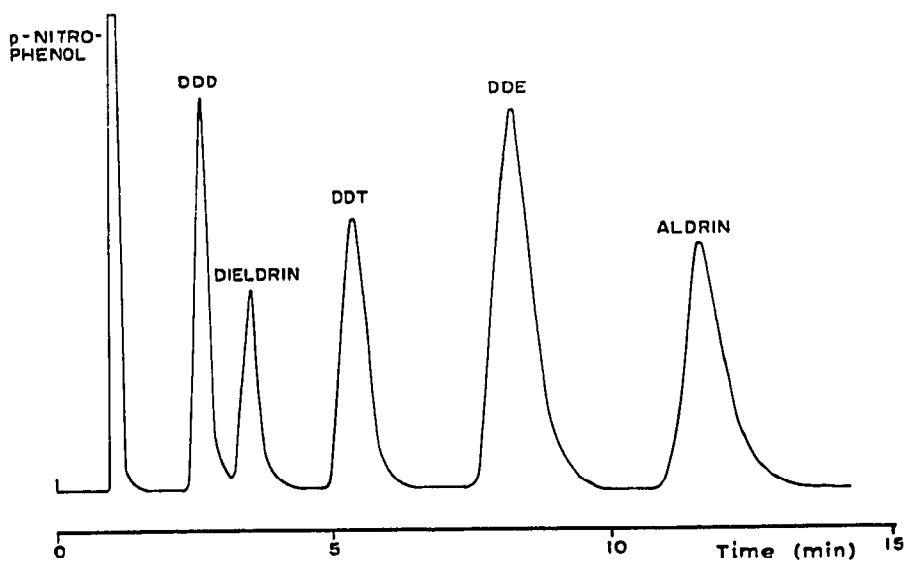


Fig. 4. Separation of a model mixture on a 10 cm  $\times$  4 mm column. Packing material and eluent as in Fig. 3.

transfer in the stationary liquid. This predominance disappears on the addition of a small amount of a polar compound to the carrier, as a result of the solvation of the polymer by the polar modifier. The influence of an apolar modifier in a polar carrier liquid was observed for reversed-phase chromatography: a strong decrease of  $H$  values and a surprising increase of  $k'$  values resulted from the addition of 1% iso-octane to a methanol-water eluent (Fig. 2). Examples of the separation of some model mixtures are given in Figs. 3 and 4. The  $H/u$  vs.  $u$  curves are comparable to those reported for other column packings (Fig. 5). The materials prepared are very suitable for use in fast analysis work.

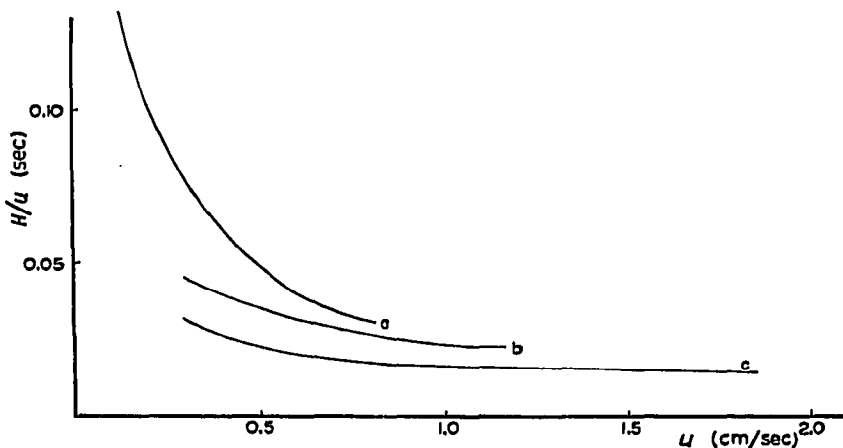


Fig. 5. Comparison of  $H/u$  vs.  $u$  curves for some packing materials. a = Hyflo Super Cel, 7–11  $\mu\text{m}$ , coated with an iso-octane-water-ethanol mixture,  $k' = 3.4$  (ref. 11); b = Hyflo Super Cel with a bonded phase prepared from  $\beta$ -(3,4-epoxycyclohexyl)ethyltrimethoxysilane,  $k' = 2.3$ ; c = silica gel, 5–10  $\mu\text{m}$ ,  $k' = 12.0$  (ref. 13).

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